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14. ABSTRACT
A method for constructing nanoscale complexes from peptides is reported, which uses branched coiled coils to create figures with specified geometry and structure. These structures can be used as vertices for programmed assembly of novel arrays, lattices and materials; the arms in our constructs are formed from alpha helical coiled coils with two or more strands. Demonstration of the design principle has been achieved. Connection of vertices to generate higher order structures is being explored, using a system for linking units that retains specific interactions between the arms.

15. SUBJECT TERMS
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FINAL REPORT

GRANT NUMBER: **N00014-02-I-0125**

PRINCIPAL INVESTIGATOR: Neville R. Kallenbach

INSTITUTION: New York University

GRANT TITLE: New System for Protein Based Nanotechnology

AWARD PERIOD: 15 November 2001-30 September 2003

OBJECTIVE:
To adapt coiled coil alpha helical proteins that normally form linear complexes to create branched structural units, or vertices, that enable multiple units to undergo programmed assembly to form spatial arrays or lattices of closed units.

APPROACH:

Coiled coils are helical proteins constructed from repeats of a seven amino acid sequence pattern, a heptad. The strategy we follow is to select complementing sets of heptads that favor selective dimer formation between each pair of arms in the molecule. Rules for complementary interaction specify two types of heptad, acidic ones (A) and basic ones (B)[1]: A type heptads repel A's and attract B electrostatically; B types repel B and attract A. The arms of our complexes consist of 3-5 adjacent heptads with a unique sequential arrangement of the two types. For example three peptides having the arrangements AAABAA, BBBABA, and BABABB will form a three-way branched peptide junction provided the orientation of sequences in one arm leads to an antiparallel coiled coil. Orientation is controlled by positioning polar Asn side chains at appropriate sites in the normally nonpolar core of a heptad[2].

ACCOMPLISHMENTS:

We have established the principle of our design using two sets of constructs shown in Scheme 1:

Set I:

pY01: **IAKLEAE NAQLEAE IAQLEAE-GS-IAKLEWE NAQLEAE IAQLRKR**

pY02: IAELRARNQALRARIAQLRKR-SG-LAKLEAENAQLRKRLAQLEKE

pY03: **RKRLAQLEAELAQNRQRLAQL-SG-IAELRARNQALRKRIAQLEAE**

Set II:

pY04: **SAIEALRAR NAQLEAE IEACERE-GG-IEALRRR NAALRWR IAQCEAE**

pY05: **SAIEALEAE NAELRRR IAACRAR-GG-LEALRRR NEALERE LEALERE**

pY06: **RARLAQL RRRLAEN ERELAEL-GG-IEALERE NEALERE IAACRWR**

Scheme 1. Sequences of two sets of three peptides designed to form three armed branched structures with each arm of the branch composed of a coiled-coil alpha helical dimer.

Based on mixing experiments using CD spectroscopy, the first set of peptides formed a trimeric complex that melted cooperatively (Fig. 1).

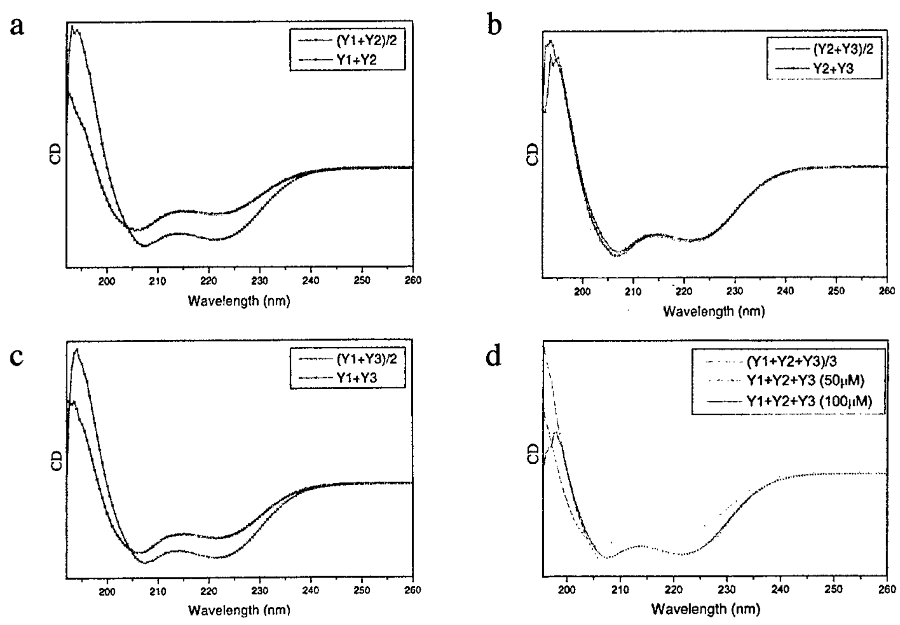


Fig. 1. CD analysis of mixtures of the peptides of Set I at 5°C. (a) Mean of CD of Y1 and Y2 relative to (Y1+Y2). (b) Mean of Y2+Y3 relative to Y2+Y3. (c) Mean of Y1and Y3 relative to (Y1+Y3). (d) Mean of spectra of Y1,Y2 and Y3 relative to equimolar Y1+Y2+Y3.

To enhance the stability we introduced disulfides to covalently join the helical arms of the complex. Evidence from CD, sedimentation equilibrium and gel electrophoresis confirmed that a trimeric complex formed again. A drawback in the design scheme of set II is that a lengthy annealing process was needed to ensure complete formation of the trimer. Our newest design obviates the need for cysteine residues within the arms, allowing formation of stable structure without extended annealing. The chains in our third set are longer and require different synthetic strategies. We have tested efficient ligation chemistry, and the new sequences incorporate one sequence that has already been cloned in *E. coli*.

RMKQLEDK VEELLSK NYHLENE VARLKKL VGERGGCGAQ LEKELQA LEKE NAQ LEWELQA LEKELAQ

RMKQLEDKVE ELLSKNYHLE NEVARLKKLV GERGGCGLEA LRRRNEALER ELEALERELE ALRRRGK

KGERELAELR ARLAQLRRRL AENERELAEL GGCGAQLKKK LQALKKKNAQ LKWKLQALKK KLAQ

Scheme 2. Sequences of Set III peptides for non-cysteine containing complexes.

In addition we have worked out a new scheme to link branched complexes specifically to create lattice like structures on the scale of nanometers (see Fig. 2).

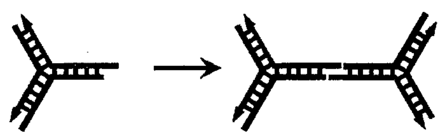
2 

Fig. 2. Assembly of two trimeric units to form a dimmer, using sticky end joining based on PNA sequences.

A persistent problem in efforts to assemble coiled coils into larger units has been the tendency of the subunits to stick and form much larger units than those we propose[3,4]. For example a method to extend coiled coils using additional matching heptads leads to fibers that are more than 50 nm thick. Our new strategy is to introduce short complementary PNA sequences at the termini of the arms[5]. These are designed to serve as sticky ends as in DNA constructs which do not associate laterally.

<u>CONCLUSIONS</u>:

Coiled-coils offer a nanoscale construction system that is superior to DNA in several respects: the chemistry of amino acids is more diverse, the association between chains can involve up to five strands, and the products are responsive to ligands, pH, solvents and temperature to be functionally useful. Relative to alternative schemes based on proteins, our constructs offer advantages of control of the valence angle at the vertex and thus positioning of the arms in space.

SIGNIFICANCE:

These preliminary results open the way to a novel and potentially useful construction kit for assembling functional structures on the nanoscale. There is great interest in the possibility of designing new materials, arrays or lattices from a "bottom up" approach to nanotechnology. We anticipate that our constructs will find application as sensors, arrays, or materials.

PATENT INFORMATION:

The Office of Industrial Liaison and Technology Transfer in the School of Medicine of NYU is exploring a filing for patent protection for applications of these structures.

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Kalinina, J., Lu, M. and Kallenbach, N.R. Branched protein structures derived from alpha helical coiled coils. Biopolymers (Submitted, 2004).